F - 201

Attachment **H**

Proposal #2001 F-201 (Office Use Only)

A. PSP Cover Sheet (Attach to the front of each proposal)

Proposal Title: Use of microbial indicators for selenium hazard assessment and for management of real-time electrical conductivity and dissolved oxygen sensor biofouling

Applicant Name: University of California, Berkeley.
Contact Name: Professor Terrance Leighton
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Amount of funding requested $485,000
Some entities charge different costs dependent on the sources of the funds, If it is different for state or federal funds list below.
State cost _____ Federal Cost _____

Cost share partners? ___ Yes ___x___ NO
Identify partners and amount contributed by each

Indicate the Topic for which yon are applying (check only on one **box)**

- ☐ Natural Flow **Regimes**
- ☐ Nonnative Invasive Species
- ☐ Channel Dynamics/Sediment Transport
- ☐ Flood Management
- ☐ Shallow Water Tidal/Marsh Habitat
- ☑ Contaminants
- ☐ Beyond the Riparian Corridor
- ☐ Local Watershed Stewardship
- ☐ Environmental Education
- ☐ Special Status Species
- ☐ Fishery Monitoring
- Fish Screens

What county or counties is the project located in?
What CALFED ecosone is the project located in? See attached list **and indicate number. Be as specific as possible** 12, 13, and 14

Indicate the type of applicant (check only one **box)**

- ☐ State agency
- ☐ Public/Non-profit joint venture
- ☐ Local government/district
- ☑ University
- ☐ Other
- ☐ Federal agency
- ☐ Non-profit
- ☐ Tribes
- ☐ Private

Indicate the primary species which the proposal addresses (check all that apply):

- ☑ San Joaquin and East-side Delta tributaries fall-run Chinook salmon
- ☑ Winter-run Chinook salmon
- ☑ Late-fall run Chinook salmon
- ☐ Spring-run Chinook salmon
- ☑ Fall-run Chinook salmon

- [ ] Delta smelt
- [ ] Splittail
- [ ] Green sturgeon
- [ ] white sturgeon
- [x] Waterfowl and Shorebirds
- [x] Migratory birds
- [ ] Other listed T/E species
- [ ] Longfin smelt
- [x] Steelhead trout
- [ ] Striped bass
- [x] All Chinook species
- [x] **All** anadromous salmonids
- [ ] American shad

Indicate the type of project (check only one box):

- [x] Research/Monitoring
- [ ] Pilot/Demo Project
- [ ] Full-scale Implementation
- [ ] Watershed planning
- [ ] Education

Is this a next-phase of an ongoing project? Yes ____ No _x___
Have you received funding from CALFED before? Yes ___ No __x__

If yes, listed project title and CALFED number _____

Have you received funding from CWIA before? Yes ____ No __x___

If yes, list CVPIA program providing funding, project title and CWIA number (if applicable):

By signing below, the applicant declares the following:

- The truthfulness of all representations in their proposal
- The individual signing the form is entitled to submit the application on behalf of the applicant (if the application is an entity or organization); and
- The person submitting the application has read and understood the conflict of interest and confidentiality discussion in the PSP (Section **2.4)** and waives any and all rights to privacy and confidentiality of the proposal on behalf of the applicant, to the extent as provided in the Section

Terrance Leighton
Print name of applicant

for Terrance Leighton
Signature of applicant

B. Executive Summary

UNIVERSITY OF CALIFORNIA, BERKELEY

BERKELEY • DAVIS • IRVINE • LOS ANGELES • RIVERSIDE • SAN DIEGO • SAN FRANCISCO

SANTA BARBARA • SANTA CRUZ

DEPARTMENT OF MOLECULAR AND CELL BIOLOGY

401 BARKER HALL
BERKELEY, CALIFORNIA 94720-3202
FAX (510) 643-5035

May 15, 2000

CALFED Bay-Delta Program Office
Suite 1155
1416 Ninth Street
Sacramento CA, 95814

Gentlemen:

Please find enclosed a proposal to the CALFED Bay-Delta Ecosystem Restoration 2001 Program entitled "Use of Microbial Community Profiles for Selenium Hazard Assessment and for Management of Real-Time Electrical Conductivity and Dissolved Oxygen Sensor Biofouling." If the proposal is selected for funding, a formal proposal will be submitted through the UCB/UCD Sponsored Projects Offices once the source of funds and awarded budget is determined.

Agricultural and wetland water districts within Bay-Delta spend hundreds of thousands of dollars each year to manage selenium laden supply water and drainage return flows. This project addresses a critical knowledge gap in ecosystem understanding and management: the role of the microbiota, which form the base of the ecosystem food web, in affecting selenium fate and transport. The project also addresses a biofouling problem that has affected a number of continuous sensors deployed by the USGS, DWR and the USBR to measure electrical conductivity and dissolved oxygen in the San Joaquin River and its west-side tributaries.

It. is expected that the project results will yield a methodology to develop more realistic concentration objectives for the SJR and its major tributaries that will both be more protective of the environment and allow agriculture to make use of the true assimilative capacity of the SJR. The current selenium objectives are neither seasonal nor site specific and hence are inherently inefficient.

The project will complement the current CALFED sponsored Real-Time Water Quality Management project, the long term goal of which is to expand forecasting to cover both selenium and boron in addition to electrical conductivity and dissolved oxygen. The sensors required for this effort are increasingly compromised by microbiota biofouling. Characterization of the microbiota responsible for these sensor failures and development of appropriate decontamination strategies is crucial to the reliability and success of the Real-Time Water Quality Management project.

We anticipate considerable interest in these projects by Bay-Delta water districts and wetland managers.

Sincerely,

for Terrance Leighton

Terrance Leighton
Professor of Biochemistry and Molecular Biology

CALFED

WATER QUALITY

Use of microbial community profiles for selenium hazard assessment and for management of real-time electrical conductivity and dissolved oxygen sensor biofouling.

Submitted by:

Department of Biochemistry and Molecular Biology
University of California
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Berkeley, CA 94720-3203

In cooperation **with** :

Lawrence Berkeley National Laboratory
Earth Sciences Division
1 Cyclotron Road
Berkeley, CA 94720

Department of Land, Air and Water Resources
University of California
Davis, CA 95616

and

Algal Research Laboratory
University of California
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US Bureau of Reclamation
2666 North Grove Industrial Drive, Suite 106
Fresno, CA 93727-1551

Department of Water Resources
3374 East Shields Avenue
Fresno, CA 93726-6913

US Geological Survey
Placer Hall, 6000 J Street
Sacramento, CA 95819-6129

May 15,2000

A. TITLE PAGE

FOCUS *AREA*. WATER QUALITY

(a) Project Title: Use of microbial community profiles for selenium hazard assessment and for management of real-time electrical conductivity and dissolved oxygen sensor biofouling

(b) Names **of** Principal Investigators:

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(c) Type **of** Organization and Tax Status: University

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(g) Participants/Collaborators in Implementation:

USGS	DWR	Panoche WD	USBR
Jerry Smithson	Ernie Taylor	Mike Gardner	Chris Eacock

B. EXECUTIVE SUMMARY

Project Title: Use of microbial community profiles for selenium hazard assessment and for management of real-time electrical conductivity and dissolved oxygen sensor biofouling

Amount Requested : $485,000 (2 years)

Name of Applicants:
Terrance Leighton — Professor of Microbiology, University of California, Berkeley
Leighton@Socrates.Berkeley.Edu, (510) 642-1620,
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Project Description:
This project has two major objectives and tasks :

1. To develop a technique for assessing site-specific selenium hazard using the microbial ecology of bacterial organisms found in the San Joaquin River and its west-side tributaries.
2. To exploit the microbial characterization developed in (1) to address a biofouling problem that has limited the use of water quality sensors for continuous sampling of electrical conductivity and dissolved oxygen in the San Joaquin Basin.

The first project task proposes the isolation, characterization, analysis and monitoring of microbial communities contained in agricultural drainage and wetland return flows generated within the Grasslands Drainage Basin on the west-side of the San Joaquin Valley and in the San Joaquin River. These will be referred to as San Joaquin River Basin (SJRB) microbial communities. The bioaccumulation and biotransformation of selenium by these SJRB microorganisms will be studied in controlled laboratory environments and free flowing aquatic systems. The processes controlling the fate and nature of selenium assimilation by microbiota will be elucidated. Advanced environmental measurement methods will be developed to determine directly the distribution and chemical species of selenium present in representative microbiota. Experimental systems will be developed to evaluate the bioavailability of microbially incorporated selenium to higher trophic levels of the food chain. Near real-time monitoring systems will be developed to "fingerprint" seasonal and treatment system changes in microbiota community structure and function. The results from this project will fill crucial data gaps in our understanding of the role of microbiota in selenium dynamics in the SJRB. The project results will also provide more realistic concentration objectives for the San Joaquin River and its major west-side tributaries that will both be more protective of the environment and allow agriculture to make use of the true assimilative capacity of the San Joaquin River. The current selenium objectives are neither seasonal nor site specific and hence are inherently inefficient. The project will complement the current CALFED sponsored Real-Time Water Quality Management project, the long term goal of which is to expand forecasting to cover both selenium and boron in addition to electrical conductivity.

The second project task proposes to use the detailed microbial community profiles developed in Task 1 to address a persistent problem that occurs in the fall of each year and affects the accuracy of real-time electrical conductivity and dissolved oxygen measurements in the San Joaquin River and its west-side tributaries. The biochemical properties of the biofilm from sensors removed from the field and taken back to the laboratory. The chemistry of the water at the monitoring location at the onset of biofouling will be determined in addition to the community profile of microorganisms associated with the water. Hence an early warning system which determines precursors to sensor biofouling and imminent failure will be developed. Further experimentation to determine effective strategies that can be used to remove the biofilm without damaging the sensor sensitivity or affecting its accuracy will be conducted in the laboratory. Effective strategies and techniques for minimizing future EC sensor failure such as the application of coatings prior to deployment, disinfection regimes, and innovative cleaning techniques will be developed. The successful conclusion of this research will enhance the quality of the electrical conductivity and dissolved oxygen data from real-time stations throughout the Bay-Delta and upper watersheds.

C. PROJECT DESCRIPTION

1. Statement of problem

a. Problem

Among the water quality issues in the San Francisco Bay-Delta region, selenium (Se) ecotoxicity represents one of the most complex problems. This is because of the extensive bioaccumulation and biogeochemical transformations of Se throughout the aquatic foodchain, which in turn dictate Se impact on the ecosystem. The recognition that the Se foodchain transfer pathway is highly dependent on a given site conditions (e.g. lentic versus lotic) further complicates the issue and necessitates the need for establishing site-specific water quality criteria for Se (see report from EPA's "Peer Consultation Workshop on Selenium Aquatic Toxicity and Bioaccumulation"). For example, despite the low waterborne Se concentrations (well below the EPA's recommended 5 µg/L limit) observed throughout the Bay-Delta, the Se body burden of an invader species of clam (Asian clam, *Potumocorbulu amurensis*) (Brown and Luoma, 1995) and the resident sturgeon species (Kroll and Doroshov, 1991) was found to be above the hazard level. The impact of such a high Se body burden in sturgeon, particularly in reproductive system tissue, is unclear. Chronic Se exposure may negatively impact sturgeon populations since Se is a reproductive stressor, causing tetratogenesis in bird and fish species (e.g. Ohlendorf et al., 1993; Lemly, 1993). Much less is known regarding the impact of the Se pathway on other fish species of the Bay-Delta ecosystem.

A major knowledge gap exists in our understanding of the unusual Se foodchain transfer pathway of the Bay-Delta ecosystem: namely the role of primary production by the microbial community in foodchain transfer from water to top predators. Bacteria and algae comprise the majority of biomass in the Bay-Delta system (see Figure 1), however there has been no systematic data collection effort to determine the effects of these communities on Se fate and transport. In particular, data relevant to the effects of primary producers on Se biodynamics (e.g. the conversion of selenate into selknoamino acids, elemental selenium, sedimentary selenite, etc.) is severely lacking. Recognition of this knowledge gap represents one consensus opinion in the aforementioned EPA report. Therefore, any change in water quality (resulting from different management practices) of source waters that significantly alters the microbial community, could lead to changes in the Se foodchain transfer pathway and its ecological impact. These effects are well illustrated by the observed changes in the phytoplankton community (USGS), Asian clam invasion (Carlton et al., 1990), and Se bioaccumulation in Asian clam and sturgeon.

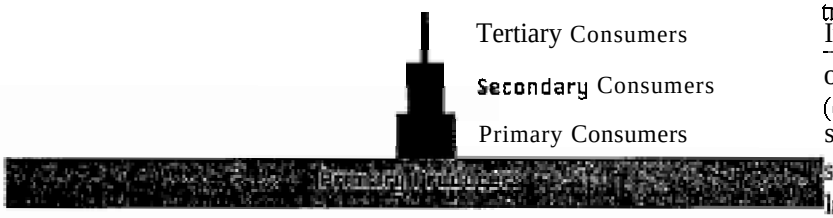


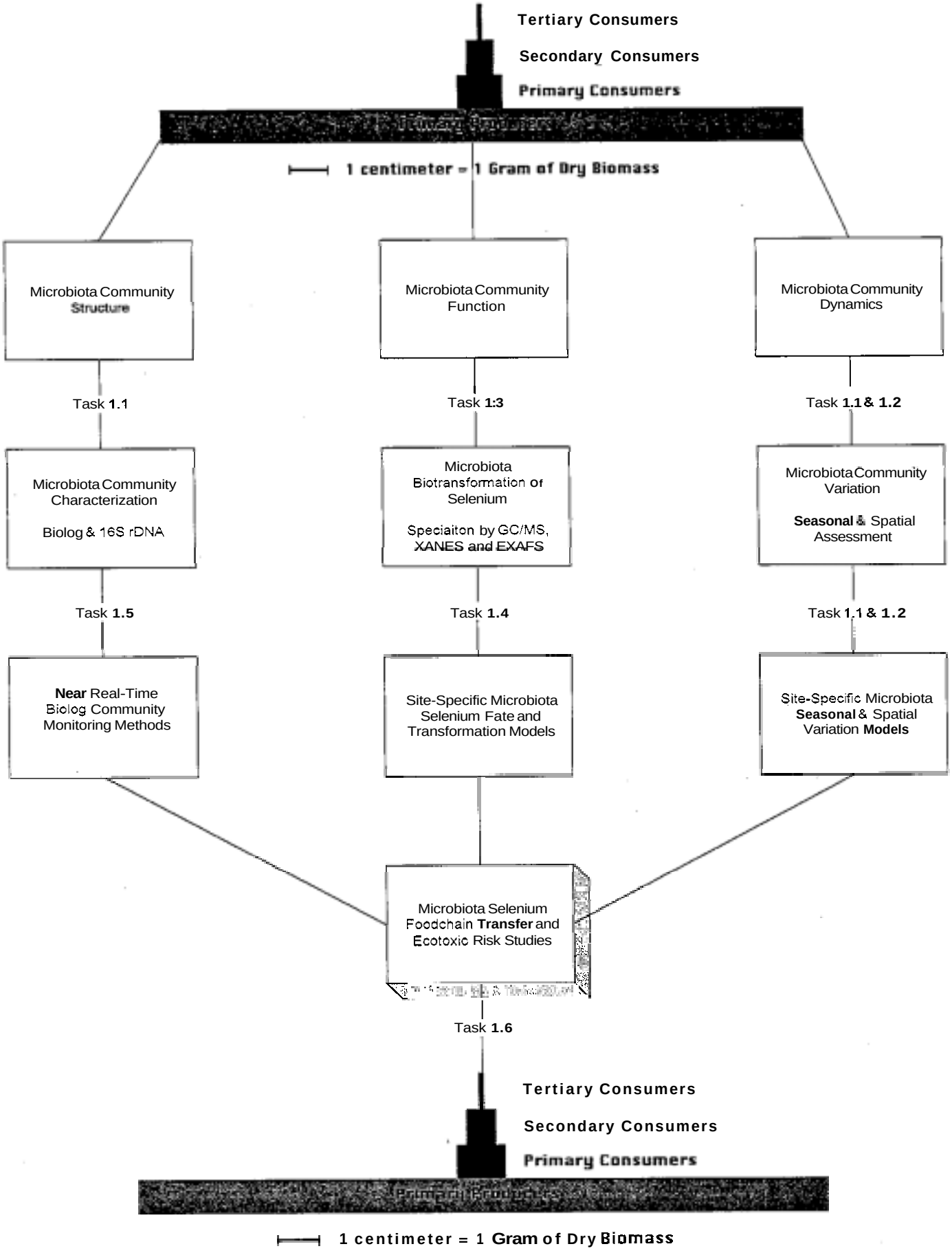
Figure 1 : Generalized foodchain transfer pathway

In Task 1 of this proposal, we will investigate how Se biotransformation mechanisms alter microbial biomass foodchain transfer characteristics and therefore, ecotoxic risk. New tools for near real-time monitoring of SJRB microbial communities will be developed. This information is critical to assessing the biological assimilatory capacity for Se in the San Joaquin River, which is in tmn needed for managing Se discharge from the San Luis Drain on a real-time basis (CALFED Project D252). In addition, the knowledge gained from this study will help guide the management of the demonstration facility for bacterial Se removal from agricultural drain waters at the Panoche Water District (Firebaugh, CA) (CALFED Project B273). These new data will complement the ongoing foodchain studies in fish species of the Delta (CALFED Project B103) and in aquatic birds of the San Joaquin Watershed (Dr. M. Fry, funded by UC Salinity/Drainage program).

In Task 2 techniques to combat biofouling of real-time sensors will benefit CALFED Projects D252 and B273 as well as two new projects, namely the Grassland Water District real-time salinity management project and the DWR project which is addressing dissolved oxygen depletion in the Stockton Deep Water Channel.

b. Conceptual model

Task 1 : The methods used to assess the selenium removal capabilities of microorganisms and microbial communities isolated from Mud Slough and SJR real-time monitoring sites (Task 1) are described below. A SJRB culture collection will be established containing representative isolates from each of the monitoring sites. Biolog and gene sequencing methods (see following sections) will be used to identify these organisms. Representative isolates' will also be used to produce well characterized biomass for Se feeding and foodchain transfer studies.


Tertiary Consumers
Secondary Consumers
Primary Consumers
1 centimeter = 1 Gram of Dry Biomass
Microbiota Community Structure
Microbiota Community Function
Microbiota Community Dynamics
Task 1.1
Task 1.3
Task 1.1 & 1.2
Microbiota Community Characterization
Biolog & 16S rDNA
Microbiota Biotransformation of Selenium
Speciaiton by GC/MS, XANES and EXAFS
Microbiota Community Variation
Seasonal & Spatial Assessment
Task 1.5
Task 1.4
Task 1.1 & 1.2
Near Real-Time Biolog Community Monitoring Methods
Site-Specific Microbiota Selenium Fate and Transformation Models
Site-Specific Microbiota Seasonal & Spatial Variation Models
Microbiota Selenium Foodchain Transfer and Ecotoxic Risk Studies
Task 1.6
Tertiary Consumers
Secondary Consumers
Primary Consumers
1 centimeter = 1 Gram of Dry Biomass

In collaboration with colleagues at the EXXON Corporate Research and at the Stanford Synchrotron Research Laboratory (SSRL) we have developed X-ray Absorption Spectroscopy (XAS) techniques to the ***in situ*** characterization of selenium fate and transformation in aquatic microbial ecosystems. The goal of the project has been to speciate selenium contaminants incorporated into biological sinks to identify selenium valence transformation and bioimmobilization mechanisms in natural environments. There is an urgent need for innovative environmental measurement technologies that are capable of ***in situ*** condensed phase toxic metal speciation. The XAS techniques pioneered at SSRL have the potential for ***in situ*** speciation of toxic metals, such as selenium, in sediments and microbia1 biomass at ppm concentration levels, without physical or chemical manipulation of the sample. XAS spectra reflect the wavelength-dependent intensity reduction of the incident beam when it passes through a sample. Inner shell electrons of the absorbing atoms, excited by X-ray photons to the continuum and interacting with neighboring atoms, cause a modulation of the absorption coefficient. The fine structure characterizing the spectra, called Extended X-ray Absorption Fine Structure (EXAFS) and X-ray Absorption Near Edge Structure (XANES) can be used to determine the short range order surrounding specific species and to obtain chemical bonding information. EXAFS refers to the sinusoidal variation of the X-ray absorption coefficient as a function of X-ray photon energy. Oscillations in the post edge region arise from back-scattering of the emitted electron wave by neighboring atoms. The demonstration by Pickering and coworkers (***Environ.*** *Sci. Technol.* 1995, 29, 2456) of the ability of XAS to speciate toxic metals, such as Se, ***in*** *situ* has created a powerful new tool for the analysis of undisturbed metal species in native environmental samples.

The goals of Tasks 1.3 and 1.4 are to exploit XAS monitoring tools to characterize the biotransformed and bioincorporated Se species that are found in microbial communities isolated from San Luis Drain, Mud Slough and SJR real-time monitoring sites. These data are crucial to identifying the mechanisms, sinks and chemical species of Se that are associated with SJRB microbial community assimilative activity. Knowledge of the chemical species abundance and distribution of bioincorporated Se is also required for the design of pure compound and biomass foodchain transfer studies (Task 1.6).

Biolog, Inc. a California Corporation, has developed an unique automated system for fingerprinting, tracking and classifying pure cultures and microbial communities from a variety of environmental samples. Communities of microbes can be directly inoculated into Biolog's 96 well Microplate test panels.

Figure **2** Biolog Microplate

After incubation a "metabolic fingerprint" characteristic of that community is obtained by scanning the Microplate with an optical reader system. The fingerprints have been shown to be both unique and reproducible. These fmgerprinting patterns allow the near real-time analysis of ecological systems and the detection of detrimental changes at an early stage. The technology also allows modeling of the affect of seasonal and operational effects on a ecological system. Biolog's test panels incorporate a novel redox chemistry to perform carbon source utilization tests for bacterial identification and fingerprinting. The chemical sensor responds to the process of metabolism (i.e. respiration) rather than to the metabolic by-products (e.g. acid). Thus, Biolog's chemistry is universal, greatly simplifying the testing process and unifying microbial identification under one single chemistry. The components of Biolog's chemistry are prefilled and dried in 96-well microplates containing 95 different carbon sources. The resulting 95-test coloriturbidity patterns provide high-resolution identification at species and subspecies levels. The identification procedure is simple and fast. Bacteria or environmental samples are inoculated into the Microplate. This takes about one minute. After incubation for either **4** hours or overnight the resulting pattern is read either with Biolog's automated Microstation instrument or by eye. Visual reading is easy because only one color (purple) is involved. Because quantitative interpretation of color reactions is imprecise by eye, the automated Microstation is required for high resolution studies.

Figure **3.** Biolog Microstation

The Microstation Systemallows the user to perform ecological analysis and also identify organisms included in Biolog's database of over 1100 species of microorganisms. We have utilized the Biolog Microstation for the analysis of ecological samples from sediments, water, and wastewater treatment systems. The Microstation provides a superior, cost-effective alternative to labor-intensive conventional microbial identification methods such as strips or panels. The 95 carbon-source tests in the Biolog Microplate are automatically read and interpreted by the Microstation instrumentation. Pure culture isolates are then identified in seconds from Biolog's extensive database of over 1,100 species/groups -- a database many times larger than strip or panel-based identification systems. Species identifications appear on the computer screen within seconds, along with biotype patterns, a list of closely related species, and other useful statistics. Intelligent software compensates automatically for different coloriturbidity

intensities, eliminating the subjectivity of visual interpretations. The software allows users to create custom databases for the analysis of unknown species or microbial community fingerprints. Cluster analysis can be performed with graphic output in the form of dendrograms, two-dimensional plots, and three-dimensional plots.

The inability to characterize *in situ* microbial communities rapidly and economically has been a severe barrier to the efficient optimization and control of biological treatment processes and the management of aquatic ecosystems. Successful development of a functionally based high-throughput methodology for the characterization of microbial communities, the community-level physiological profile (CLPP), provides a critical enabling technology for minimizing the environmental impacts of Se loading. CLPP can provide significant benefits in terms of cost savings, throughput, and sensitivity over existing baseline technologies for microbial treatment system monitoring (e.g., direct microscopic observation, plate counts, biochemical measurements, etc.). CLPP is also useful for microbial community monitoring in association with the adaptive management of ecosystem performance. Garland and Mills (Garland, J. and A. L. Mills. 1991 first conceived the community-level physiological profile (Classification and Characterization of Heterotrophic Microbial Communities on the Basis of Community-Level Sole-Carbon Source Utilization. Appl. Environ. Microbiol. 57:2351-2359) to distinguish microbial communities from diverse habitats, and along gradients within a given habitat. This assay involves inoculating whole communities from environmental samples into Biolog microbial identification system GN (Gram-negative) microtiter plates and evaluating respiration of ninety-five different sole carbon sources by an automated microtiter plate reader and associated data analysis software. The multivariate dataset is analyzed by principal components analysis (PCA) which extracts major trends in the dataset and allows distinctions between communities that can be correlated with the original variables [sole carbon sources] and perturbations of the ecosystem.

We have demonstrated the power of the CLPP technology is a three-year pilot study of the microbial community residing within a selenium algal-bacterial treatment system. This project had *two* goals: (i) the development of monitoring tools to assess microbial community adaptation and adjustment to variations in treatment system operating parameters; and (ii) the use of CLPP as a high-throughput screening system to engineer the microbial community metabolically for optimal toxic metal bioremediation. CLPP has proven to be a very sensitive and incisive tool for assessing the integrity and functionality of the treatment system microbial community. CLPP has been used to track a treatment system upset that was caused by an alteration in the food to mass ratio of the wastewater feed stream. This perturbation resulted in significant negative impacts on treatment system nitrification and settling parameters. The surprising result from studies at the treatment system is that microbial communities have very simple and diagnostic CLPP signatures. We interpret these results to mean that these communities are highly differentiated metabolic specialists, rather than metabolic generalists who would be expected to exhibit far more complex CLPP signatures than we have observed. The "Normal" CLPP pattern has been stable over three years of treatment system monitoring.

Over the past fifteen years, molecular tools have forever changed studies of microbial evolution and taxonomy by providing more robust phylogenetic frameworks based upon objective genetic criteria. Comparisons of small subunit ribosomal rRNA sequences (16s-like rRNAs) are particularly valuable for phylogenetic inference. The small subunit rRNA database comprises the largest collection of sequences that share a common ancestry (presently over 1400 microbial entries). These genes are present in all cells where they perform similar functions. Since ribosomal RNA genes do not undergo lateral transfer between organisms, molecular trees inferred from comparisons of their nucleotide sequences accurately depict the historical evolution of their corresponding genomes.

Biolog will be the primary tool used for the classification of microorganisms isolated from SJRB monitoring sites in the San Luis Drain, at Mud **Slough** and SJR monitoring sites (Tasks 1.1 and 1.2). Biolog CLPP will be used as a near real-time monitoring tool to fingerprint microbial community signatures at these sites (Task 1.5). 16S rRNA gene-sequencing methods will be used as required validating Biolog identification and in cases where Biolog is unable to accurately assigning a genus and species. CLPP fingerprints will be developed for both sites that characterize normal and abnormal operating regimes. CLPP fingerprinting will be used for stability/recovery analysis of monitoring sites, to determine whether there is a defined recovery path, and to validate the potential of this technology for process monitoring and control:

Samples from SJRB monitoring sites will be subjected to CLPP to produce a multidimensional profile of the mixed aerobic heterotrophic community based on sole carbon source utilization in Biolog GN MicroPlates. Communities will be compared on the basis of average metabolic response (AMR), metabolic diversity, and PCA analysis of the multivariate substrate utilization profile.

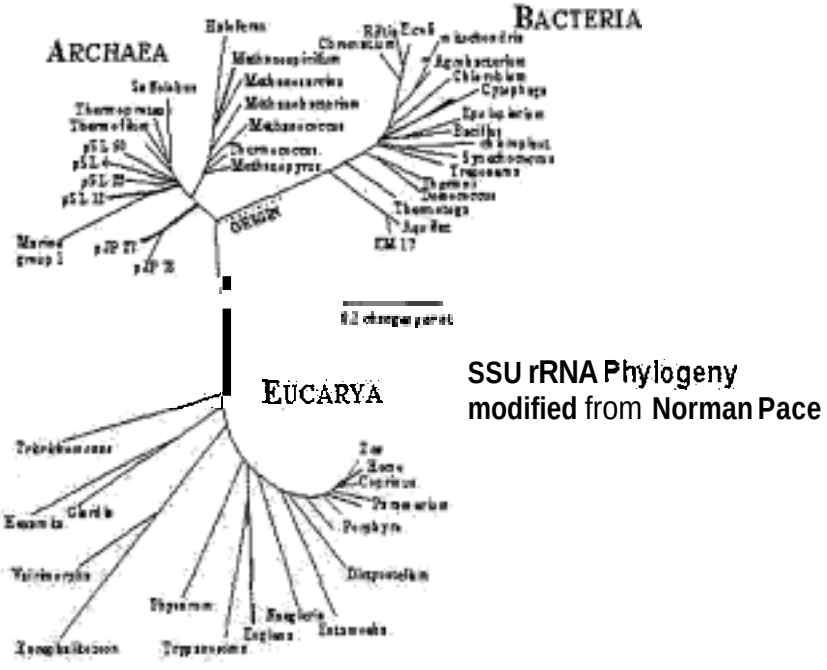


Figure 4. 16S rRNA Phylogenetic Tree of Life

Collateral data on system operating parameters will also be collected and archived. The response of communities to changes in system operating parameters will be quantitatively evaluated. Metabolic patterns of succession over the course of seasonal variations will be investigated during periods of baseline and subnormal community function. This metadata will provide a comprehensive resource for understanding normal and perturbed system operational regimes.

Two resident species of the San Joaquin River, San Luis Drain, and Delta, red swamp crayfish ***(Procambarus clarkii)*** and mosquito fish ***(Gambusia),*** will be used for the bacterial foodchain transfer experiments. Both species are ubiquitously abundant in the proposed region and serve as direct links from the primary/secondary producers to the top predators including those that are threatened (e.g. Sacramento perch, salmon, hardhead minnow, white-faced ibis, California clapper rail, pied-billed grebe, etc). Teratogenic effects have been documented for mosquito fish in Se-contaminated sites (Lemly, 1993). The field abundance of the two species makes it practical to collect samples from relevant sites, which provides the unusual opportunity to relate results from a laboratory feeding study to field observation. Both species are also polytrophic in terms of their diets, which makes them excellent organisms for examining integrating effects of Se derived from various food sources ranging from detritus, producers, to primary and secondary consumers. Moreover, both species are convenient to rear in the laboratory, and only require several months to become reproductive from larval stage, which will greatly facilitate the feeding studies.

Crayfish will be raised in the UCD flow-through system from the egg to reproductive stage, while mosquito fish will be raised from larval to reproductive stage. For the 1st week of development, larval mosquito fish will be fed brine shrimp nauplii and trained to feed on prepared food as described below. The Se-contaminated food will be prepared from bacterial biomass grown from different Se treatments or pure selenium species, encased in gelatin, and graded into appropriate sizes for feeding from larval to adult stages. The gelatin feeding method has been tested successfully on crayfish and is expected to work for mosquito fish as well. The Se treatments will be adjusted such that the bacterial biomass is respectively dominant in selenomethionine, selenocystine, and selenium element (as determined by XANES and/or GC-MS). If necessary for nutritional balance, casein and/or brine shrimp will be included in the feed. Samples will be taken at larval, pre-, and post-reproductive stages, freeze-dried, and pulverized for Se analysis. When practical, target organs including hepatopacreas/egg mass of crayfish and liver/gonad of mosquito fish will be analyzed separately, which should provide a closer link to ecotoxic risk. Se analysis will include total Se measurements by the fluorescence method (Fan et al., 1998a), free and protein-bound selenoamino acids by GC-MS (Fan et al., 1998a&b) or **XANES,** and elemental selenium by **XANES.**

These studies (Task **1.6)** will provide unique information on how various bioconcentrated Se metabolites are assimilated from food sources and in turn metabolized in consumers, particularly into the proteinaceous fraction which is generally recognized as the most ecotoxically relevant pool (see **EPA's** Peer Consultation report). The comparison of Se bioavailability to higher trophic levels from pure compounds and biomass incorporated species will allow a data-driven assessment of the ecotoxic risk posed by Se incorporated into microbial sinks.

Task 2 : Sensor biofouling is a problem in a number of environmental and industrial settings. The precursors to biofouling are not completely understood in all circumstances. Biofouling can occur as a result of a sudden change in the chemistry of the water being monitored or in the food supply available to certain strains of microorganism. The

microbial ecology of the water may be the greatest factor related to the onset of sensor biofouling. A biofilm consists of microbial cells (algal, fungal, or bacterial) and the extracellular biopolymer they produce. It is bacterial biofilms that are of most concern in many water systems, since they are generally responsible for equipment fouling. The more nutrients available in the form of useable organic carbon, the greater the diversity and numbers of organisms that can be supported in biofilm communities. In free flowing aquatic systems, algal biofilms can **also** be a concern. Not only will algal biofilms foul distribution equipment, but algae will also provide nutrient (organic carbon) that will help support the growth of bacteria and fungi. Algae do not require organic carbon for growth but instead utilize CO_2 and the energy provided by the sun to manufacture carbohydrate. **A** water system with little organic carbon can generate considerable biomass through the growth, dispersal and decomposition of algal cells.

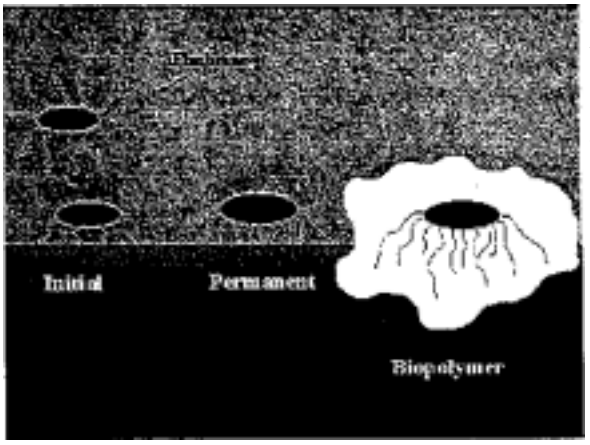


Figure 5.

Microorganisms can be found in both bulk phase and on water system and equipment surfaces. Bacteria attach to surfaces by proteinaceous appendages referred to as fimbriae (Figure ***5)***. Once a number of fimbriae have "glued" the cell to the surface, it makes detachment of the organism very difficult. One reason bacteria prefer to attach to surfaces **is** the organic molecules adsorbed there provide a higher local nutrient concentration. Once attached, the organisms begin to produce material termed extracellular biopolymer **or** "slime". The amount of biopolymer produced can exceed the mass of the bacterial cell by a factor of 100 or more. The extracellular polymer produced may tend to provide a more suitable protective environment for the survival of the organism.

Xanthan Gum from *Xanthamonas campestris*

The extracellular biopolymer consists primarily of polysaccharide and water. The polysaccharides produced vary depending on the bacterial species but are typically made up of repeating oligosaccharides, such as glucose, mannose, galactose, xylose, and others. One example of a bacterial-produced biopolymer is xanthan gum (Figure 5), produced by *Xanthamonas campestris*, a microorganism frequently isolated from San Joaquin Valley drainage sites (Leighton et al., unpublished data). Gellation of some biopolymers can occur upon addition of divalent cations, such as calcium and magnesium. The electrostatic interaction between carboxylate functional groups on the polysaccharide and the divalent cations results in a bridging effect between polymer chains. Bridging and crosslinking of the polymers help to stabilize the biofilm, making it more resistant to shear.

Figure *6.*

Once bacteria begin to colonize surfaces and produce biofilms, numerous problems begin to arise, including reduction of transfer efficiency, fouling, corrosion, and scale (Figure 6). When biofilms develop in low flow areas, they may initially go unnoticed, since they will not interfere with flow or equipment operational efficiency. Over time, the biofilm becomes more complex, often with filamentous development. The matrix provided will accumulate debris that may impede or completely block flow and performance of sensitive equipment. Deposits in the form of biofilm and biofilm with entrapped suspended debris are common, but biofilms may also lead to the formation of mineral scales. Calcium ions are fixed into the biofilm by the attraction of carboxylate functional groups on the polysaccharides. In fact, divalent cations, such as calcium and magnesium, are integral in the formation of gels in some extracellular polysaccharides. If we can imagine these calcium ions being fixed in place by the biofilm at a critical surface, then it would make them more readily available to react with carbonate **or** phosphate anions that are present. This would then provide nucleation or crystal growth sites that would not normally be present on a biofilm free surface. Additionally, biofilms may entrap precipitated calcium salts and corrosion by-products from the bulk water that will act as crystal growth sites.

Growth of bacteria on surfaces in water systems can lead to significant deposit and corrosion problems. Once this is understood, then the importance of controlling biofilms becomes quite clear. Biofilms can be controlled through the use of microbicides, biodispersants, and by limiting nutrient. Microbicides, both oxidizing and nonoxidizing, can be effective in overall biofilm control when applied properly. The oxidizing microbicides, such as chlorine, bromine, chlorine dioxide, and ozone, can be extremely effective in destroying both the extracellular polysaccharide and the bacterial cells. When using oxidizing microbicides, one must be sure to obtain a sufficient residual for a long enough duration to effectively oxidize the biofihn. Unfortunately, the corrosive nature of the oxidizing microbicides and their undesirable effects on sensitive equipment and surfaces may limit their usefulness. Low residual oxidant levels may significantly reduce planktonic counts but may not be sufficient to control biofilm. The level of oxidant and duration required will vary from system to system. It is generally more effective to maintain a higher residual for a duration of several hours than it is to continuously maintain a low residual. Continuous low-level feed may not achieve an oxidant level sufficient to oxidize the polysaccharide and expose the bacteria to the oxidant. Another misconception is with the use of chlorine at alkaline pH (> 8.0). It is often thought that chlorine is ineffective for controlling microorganisms at elevated pH. Certainly, the hypohalous acid form of chlorine (HOCl) is more effective at killing cells than the hypohalite form (OCl-). However, the hypohalite is actually very effective at oxidizing the extracellular polysaccharide and the proteinaceous attachment structures. Therefore, the use of chlorine in alkaline conditions can still be extremely effective. This is especially true when combining chlorine with bromine or with a compatible nonoxidizing microbicide such as a polyquat. When this is done, one can achieve both oxidation of the extracellular material and sufficient kill of the microorganism.

Nonoxidizing microbicides are also effective in controlling biofihn formation and development. Effective control is greatly dependent on frequency of addition, level of feed, and resistance of the incumbent population to the product being fed. Control cannot generally be achieved by once-a-week additions as is common in "full service" applications. Typical application for effective control may include a slug addition of product 2 to 5 times a week. As with oxidizing microbicides, frequency and dosage will depend on the system conditions. It is generally most effective to alternate nonoxidizing microbicides at every addition to ensure broad spectrum control. Most nonoxidizing microbicides will have little effect in destroying the extracellular polysaccharide found in the biofilm. However, many microbicides may be able to penetrate and kill bacteria found within the biofihn. Combining the use of nonoxidizing and oxidizing microbicides is a very effective means of controlling biofilm. When using a nonoxidizing microbicide in conjunction with an oxidizing agent, there should be no residual oxidant present in the system at the time of addition. Sufficient time should be allowed for the nonoxidizing microbicide to work before resuming oxidant feed unless an oxidant compatible microbicide is being used (i.e., polyquat).

Biofilm control programs can be made more effective through the utilization of a biopenetrant/dispersant product. Products that penetrate and loosen the biopolymer matrix will not only help to slough the biofilm but will also expose the microorganisms to the effects of the microbicide. These products are especially effective when dealing with systems that have a high TOC loading and a tendency to foul. These products are typically fed in slug additions prior to microbicide feed. Low-level continuous feed may not be as effective, since it often takes a certain threshold amount to produce the desired effect. Recent developments in biodispersant technology is making this approach more effective and popular than ever before. Enzyme technologies that will break down the extracellular polysaccharides and degrade bacterial attachment structures (fimbriae) are currently being developed and patented. These technologies, although expensive, may provide biofilm control where microbicide use is environmentally restricted or provide a means of quickly restoring fouled sensitive equipment to a clean, efficient operable state. The importance of biofilm control is crucial in any real-time monitoring project. It is the fundamental basis for controlling a high percentage of sensor performance problems in free flowing aquatic systems. Once the fundamentals of biofilm development and control are understood, effective treatment strategies can be developed and implemented.

Algal and bacterial agents responsible for biofouling, identified using techniques described in Task 1 will be subjected to chemical, biochemical and physical techniques to effectively control and eliminate the problem when it typically occurs (in the late summer of each year in the case of electrical conductivity sensors). Although there is a considerable literature on techniques of disinfection, chemical treatment and physical removal to combat biofouling these techniques have not been evaluated for potential damage to sensitive electronic and electrochemical sensors nor have they specifically targeted to specific algal and bacterial species, since these data very rarely exist..

c. Hypotheses being tested

Hypothesis/Question to be Evaluated	Monitoring Parameter(s) and Data Collection Approach	Data Evaluation Approach	Comments/Data Priority
1.1 Characterization of microbial communities within the SJRB can be used to estimate primary producer contributions to selenium flux and transport in the SJR.	Assessment of microbial community structure and dynamics.	Determination of microbial community genus and species composition and seasonal variations in microbial community structure. Determination of microbial isolate and community selenium removal kinetics.	High-need data required to assess the role of primary producers in selenium dynamics.
1.2. Characterization of microbial communities within the **SJRB** to estimate primary producer contributions to selenium bioassimilation and bioaccumulation within the SJR.	Assessment of microbial isolate and community bioassimilation and biotransformation of soluble selenium species.	Determination of the kinetics and extent of soluble selenium species assimilation and valence transformation by microbial isolates and communities from the SJRB.	High-need data required to assess the role of primary producers in selenium bioaccumulation and biotransformation.
1.3. Community Level Physiological Profile methods can be used to monitor microbial communities in near real-time.	Biolog monitoring of SJRB and Panoche Biotreatment Plant microbial communities.	CLPP signature databases analysis of normal and abnormal system operational fingerprints.	Near real-time monitoring microbiota database for use in adaptive management of selenium flux.
1.4. Characterization of food chain transfer of microbiota incorporated selenium can be used to estimate primary producer contributions to selenium ecotoxic risk of withii the	Assessment of food chain transfer of microbial isolate and community bioaccumulated selenium species to swamp crayfish and mosquito fish.	Determination of the kinetics and extent of selenium species accumulation and valence transformation by higher trophic level receptors indigenous to the SJRB.	High-need data required to assess the biomagnification and ecotoxic risk of primary producer incorporated selenium to fish and invertebrates in the SJRB.
2.1. Characterization of biofilm and determination of biochemical and biophysical properties.	Scanning electron microscopy for microbe identification. Biolog to identify bacterial species and characteristics.	Comparison with databases of known physical and chemical properties.	Experimentation with effective biocides following identication.

d. Adaptive management

The project management plan is consistent with the concept of adaptive management which is to design a set of experiments in a scientifically sound manner to converge on the best possible solution **or** technique. In the case of this proposal the practices of good science will suffice to achieve project objectives. Even with improved techniques for selenium risk assessment it will take an adaptive process for institutions to evolve to take advantage of the new paradigm and for statutes to be written to provide regulatory agencies with the authority to manage selenium loads using microbial sensors. This is consistent with the adaptive management process.

e. Educational objectives

This project will be conducted at two University campuses and at a National Laboratory. Students from these institutions will be employed to work on these grants providing an educational opportunity for these individuals. In addition seminars and technical papers will result from the research allowing further dissemination of the information.

2. Proposed scope of work

a. Location and geographic boundaries

The study sites are located within Merced County. The project area includes the San Joaquin River and its west-side tributaries that drain through Mud Slough and Salt Slough.

b. Approach

We propose to undertake the following studies :

Task 1 : Characterize microbial community structure, function and dynamics in Mud Slough and the San Joaquin River. Mud Slough is the major conveyance of salt and selenium to the San Joaquin River since the implementation of the Grassland Bypass project in 1996, which diverted agricultural drainage into the San Luis Drain. The selenium objective of 5 ppb is continuously exceeded in Mud Slough downstream from the terminus of the San Luis Drain. The CALFED goals of the following experiments are designed to formulate a more realistic seasonal, site-specific selenium objective for Mud **Slough.**

1.1 Assess microbial community structure and seasonal variation by direct isolation of microbiota and subsequent classification using Biolog and 16S rRNA gene-sequencing methods.

1.2 Assess the effects of drainage system operating parameter variations on microbial community structure.

1.3 Assess the ability of representative isolates and communities to assimilate and biotransform selenate and selenite into organic, elemental and volatile selenium species.

1.3.1 Advanced environmental measurement methods including GC/MS, XANES and EXAFS will be used to determine selenium fate and chemical species.

1.4 Assess the effects of drainage system operating parameter variation on microbial community assimilation and biotransformation of selenate and selenite into organic, elemental and volatile selenium species.

1.5 Develop near real-time drainage system microbiota monitoring methods by using Biolog profiling of microbial community metabolic signatures at Mud **Slough** and San Joaquin sites.

1.5.1 Correlate variations in Biolog community level physiological profiles (CLPP) with seasonal fluctuations in the drainage system environment.

1.5.2 Develop a CLPP database documenting microbial community signatures typical of variations in drainage system operating modes.

1.5.3 Correlate CLPP signature data with real-time monitoring project data to develop selenium adaptive management strategies.

1.6 Assess the ecotoxic risks of bioincorporated selenium species by pure compound and biomass foodchain transfer studies with two resident Bay-Delta species, red swamp crayfish ***(Procambarus clarkii)*** and mosquito fish ***(Gambusia)*** receptors.

Task 2 :

2.1. Examine the biofihn and determine the physical properties of the biofilm from an autopsy of failed EC sensors removed from the San Luis Drain. Determine effective regimes that can be used to remove the biofilm without damaging the sensor sensitivity or affecting its accuracy.

2.2 Determine the microbial ecology of water column at each of three monitoring locations : Site B (San Luis Drain between Check 1 and the terminus), Site D on Mud Slough (1/4 mile downstream from the San Luis Drain terminus) and Crows landing on the San Joaquin River. Culture major algal and bacterial species.

2.3 Grow biofilms on electrodes of the same material used in the Campbell EC sensors and determine physical and chemical properties of these biofilms.

2.4 Determine effective strategies and techniques for minimizing future EC sensor failure such as the application of coatings prior to deployment, disinfection regimes, or innovative cleaning techniques. Development of an effective maintenance and monitoring strategy to minimize site visits without compromising data accuracy.

c. Monitoring and Assessment Plans

The monitoring and data gathering required for successful completion of this project will complement the existing compliance monitoring program being undertaken by the cooperating agencies in the SJRB and the CALFED-sponsored "Real-Time San Joaquin River Water Quality Management" project, being undertaken by the SJRMP Water Quality Subcommittee. A Quality Assurance Project Plan will be developed for the analytical procedures undertaken as part of this project and filed with CALFED in the first 3 months of the project.

d. Data handling and storage

Data gathered by the individual researchers will be stored in EXCEL spreadsheets and ACCESS databases that will be supplied to CALFED together with the final project report at the conclusion of the study. Because this study has many collaborators in a wide range of agencies data will be readily accessible by these individuals once the appropriate data quality checks and laboratory quality assurance has been completed to accelerate progress toward development of site-specific selenium concentraion objectives and to solve the existing sensor biofouling problem.

e. Expected products and outcomes

The outcome of Task 1 will be a working methodology and set of microbial assessment tools to assist regulators in the development of site-specific selenium objectives and those regulated to assess the impact of their selenium contaminated return flows on the environment. The outcome of Task **2** will be a technique to forecast potential biofouling problems before they occur and a protocol for eliminating and managing biofouling of electrical conductivity and dissolved oxygen sensors when they occur in the late summer and fall of each year.

f. Work schedule

The proposed project will have a two year duration with the initiation of the Task 1 microbiota monitoring and pure compound foodchain studies and biofouling organism identification occurring during year 1, the continuation of microbial monitoring and biomass foodchain studies during year **2,** and the field coordination with the SJRMP Water Quality subcommittee for both Task 1 and Task **2** studies in both years 1 and *2.* Procedures for the isolation, characterization and classification of microbiota, procedures for obtaining microbiota CLPP, and advanced environmental measurement methods for the speciation of Se in biomass, will be established and validated during the first 6 months of the project and will be implemented during the project's two year term. Remedies to combat sensor biofouling will be tested in the field and coordinated with the Task **2** project cooperators in the USBR, DWR and the USGS.

The work schedule is shown in the table below. Two progress reports and one final project report will be prepared summarizing the objectives accomplished during the year and results from activities in the SJRB. Demonstrations and workshops will be conducted to disseminate results form the project and to introduce potential users to the Biolog CLPP microbiota monitoring technology.

PROJECT MONTH	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
REPORTS											X	X											X	X
TASK 1.1		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
TASK 1.2										X	X	X	X	X	X	X	X	X	X	X	X	X		
TASK 1.3						X	X	X	X	X	X	X	X	X	X	X	X	X						
TASK 1.4												X	X	X	X	X	X	X	X	X	X	X		
TASK 1.5						X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
TASK 1.6			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
TASK 2.1		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
TASK 2.2										X	X	X	X	X	X	X	X	X	X	X	X	X		
TASK 2.3										X	X	X	X	X	X	X	X	X	X	X	X	X		
TASK 2.4												X	X	X	X	X	X	X	X	X	X	X		

g. Feasibility

Over the past five years the project team has developed, tested and deployed all of the tools required for the successful completion of the proposed scope of work. Some examples of these efforts and their relevance to CALFED objectives are described below in order to establish the technical feasibility of the proposed workplan.

We have demonstrated previously that a well characterized laboratory strain of the common soil and aquatic Gram-positive bacterium, ***Bacillus*** *subtilis,* can detoxify soluble Se by aerobic reduction to an insoluble and nontoxic form, elemental selenium (Garbisu et al., 1995; Garbisu et al., 1996; Garbisu et al., **1997,** Garbisu et al., 1999). We have developed a chemically defined minimal growth medium and Atomic Absorption Spectroscopy methods to quantify the assimilation of soluble selenium species (selenite and selenate) by pure microbial cultures and microbial communities. *B. subtilis* is able to grow and detoxify soluble Se at concentrations up to 400 ppm. At these high Se

concentrations, the primary biotransformed Se species is elemental Se. The Se valence transformation to nontoxic elemental selenium is not affected by a ten-fold excess of nitrate or sulfate - alternate electron acceptors which block Se reduction in anaerobic treatment systems (Garbisu et al., 1995). We conclude that soluble Se is not reduced via dissimilatory electron transport but rather via a novel detoxification system. These results indicate that the soil bacterium ***B. subtilis*** and related organisms form the basis of a very promising technology for bioremediating selenite. At lower soluble Se concentrations typical of SJRB sites - 100 ppb - ***B. subtilis*** **was** able to remove **96%** of the Se from the liquid phase (see Figure 7). These results highlight the importance of microbiota in Se bioconcentration. In these experiments the microbiota occupy 1/1000 of the bulk liquid phase volume. Hence, biomass assimilated Se is concentrated 1000X above the level found in the water column.

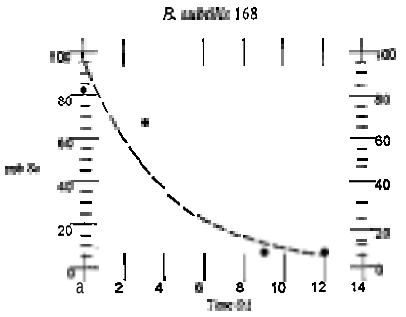


Figure **7** Selenite Removal During Growth **of** ***B. subtilis***

At these environmental Se concentrations, the primary biotransformed Se species are selenoamino acids. A high-energy carbon source, such **as** glucose, supports optimal Se reduction. These results have been used to design and demonstrate a bacterial selenium removal treatment system constructed in the Panoche Water District in collaboration with the UC Algal Research Group (CALFED Project B273).

Several hundred individual bacterial strains have been isolated from the Agatha Canal, San Luis Drain and the Panoche Water District. A majority of these isolates are Gram-negative bacteria that were able to assimilate Se with kinetics similar to well characterized laboratory strains of *B. subtilis.* At the lowest soluble Se concentration studied - 150 ppb - these isolates were able to remove >98% of Se from the liquid phase (see Figures 8 and 9).

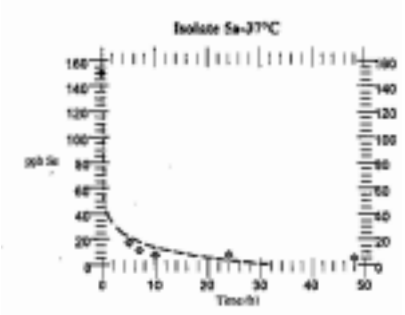


Figure **8**

Selenium Removal During Growth **of** Isolate 5a

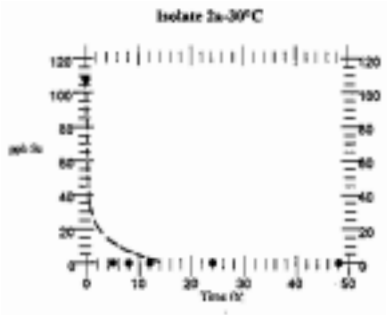


Figure ***9***

Selenium Removal During Growth **of** Isolate 2a

It is clear from these data that the microorganisms resident in the SJRB are capable of bioassimilating Se and removing it from bulk liquid phase. On-going studies are directed at taxonomically identifying the Se removing members of the SJRB microbial population (principally Gram-negative groups such as pseudomonads) and assessing the effects of seasonal SJRB and algal/bacterial Se treatment plant operation parameters on the structure, function and dynamics of these microbial ecosystems.

D. APPLICABILITY TO CALFED ERP GOALS, IMPLEMENTATION PLAN AND CVPIA PRIORITIES

a. ERP Goals and **CVPIA** priorities

Selenium (Se) entering the lower San Joaquin River (SJR) is the primary stressor. Discharge into the SJR of agricultural drainage high in Se is a serious contaminant problem in the lower SJR basin and Bay-Delta. Selenium has caused reproductive failure in sensitive fish species and developmental deformities in waterfowl and shorebirds because of its ability to bioaccumulate to levels that can be toxic to higher trophic organisms. Project benefits include: (1) generation of microbial community structure, function and dynamics data to assess impacts on selenium loading that address deficiencies in the current CALFED-sponsored real-time water quality forecasting project on the mainstem of the SJR (2) near real-time microbiota monitoring tools for use in the control of a selenium bioremoval demonstration project within the Panoche Water District; **(3)** near real-time microbiota monitoring tools **for** use in

the current CALFED-sponsored real-time water quality forecasting project on the mainstem of the SJR; **(4)** an understanding of the ecotoxic risk of bioincorporated selenium to SJR fish and invertebrate species; and *(5)* the potential for increasing the frequency of meeting SJR water quality objectives.

Spring releases of water from seasonal wetlands are discharged into tributaries of the Lower SJR. These releases, in combination with agricultural drainage that flows through the Grasslands Water District (GWD), contain varying amounts of selenium. Selenium has been identified as a stressor that leads to frequent exceedance of water quality objectives established for the San Joaquin River by state and federal agencies.

Research conducted by Grober et al. (1995) suggests that wetland drainage from the GWD could be scheduled to coincide with peak assimilative capacity in the SJR to help improve downstream water quality. In addition, increased water supply allocations under the Central Valley Project Improvement Act (CVPIA) have created opportunities to coordinate the release of seasonal wetland drainage with the assimilative capacity of the SJR. Coordinated releases will help to achieve water quality objectives and improve fish habitat in the main stem of the SJR and Sacramento - San Joaquin Delta. Improved scheduling of west-side discharges can assist in avoiding critical time periods for fish rearing and remove an important stressor leading to improvements in the San Joaquin salmon fishery. To date, however, no systematic data collection program has been undertaken to elucidate the effects of microbial communities on selenium dynamics and to incorporate these insights into real-time wetland drainage management.

Management of wetland drainage through scheduling of releases to coincide with periods of SJR assimilative capacity can help to improve SJR water quality. However, these actions may need to consider the biological impacts of changes to traditional wetland management practices. Peak assimilative capacity typically occurs between the months of January and April. This time period is often times earlier than the traditional wetland draw-down period (March-April). In particular, the response of migratory waterfowl and shorebirds to an early draw-down regime needs to be assessed to determine potential impacts to foraging rates, habitat availability, and species diversity and abundance. It is possible that early, experimental draw-down may make food sources available to wildlife without negatively affecting wetland vegetation community and plant species diversity - hence benefiting both wildlife and the SJR. This project should have considerable technology transfer value to other agencies that operate seasonal wetlands and also discharge constituents of concem to the SJR.

The project's microbiota monitoring activities will increase the understanding of factors that affect SJR water quality. This information provided by this secondary benefit could be used to assess the impact of other management practices that attempt to reduce the pollutant load into the lower SJR and Bay-Delta. Species and species groups benefiting from reductions in contaminants entering the Bay-Delta are delta smelt, longfin smelt, splittail, white and green sturgeon, striped bass, resident fish species, marine/estuarine fishes and large invertebrates, Bay-Delta aquatic foodweb organisms, and waterfowl.

Non-ecological CALFED objectives addressed by project include improving SJR and Bay-Delta water quality for agricultural, drinking water, industrial, and recreational beneficial uses. The project will provide data that will facilitate the control and timing of wetland and agricultural drainage to coincide with periods when dilution flow is sufficient to achieve CALFED water quality concentrations.

b. Relationship to other ecosystem restoration projects

The described data collection under Task 1 and Task 2 are consistent with the current CALFED-sponsored initiative on real-time water quality management in the San Joaquin River, the Vemalis Adaptive Management Program (VAMP), a multi-agency experiment to improve the San Joaquin River fishery through manipulation of tributary flows and flow release schedules and The Grasslands Bypass project, a five year experiment in the Grasslands Basin to control selenium loads discharged to the San Joaquin River. In addition Task 2 is relevant to the newly funded Grassland wetland adaptive management project for salinity loading to the San Joaquin River and the upper watershed monitoring effort being undertaken DWR to address dissolved oxygen sag in the Stockton Deep Water Channel

c. Requests for next-phase funding

The two year study described in this proposal comprises two tasks. Task 1 is a proof of concept study which may warrant further funding if the techniques developed show promise for real-time selenium water quality management and utility for the development of site-specific selenium total mass daily loads (TMDLs) for the Grassland Basin. If Task **2** is successfully concluded the techniques developed should have immediate application to biofouling of real-time electrical conductivity and dissolved oxygen sensors and hence there is unlikely to be a need for further funding.

d. Previous recipients **of CALFED** or **CVPIA** funding

Neither Task 1 nor Task 2 of this proposal have received previous CALFED or CVPIA funding.

e. System-wide ecosystem benefits

The proposed project will provide basic monitoring, decision support tools, and selenium bioremoval system information to allow managers in the SJRB to respond to the long-term challenge of improving water quality while maximizing ecosystem functions and habitat values. Information obtained through this project will be transferable and of significant value to all operators in the SJRB. The successful implementation of this combined monitoring, experimentation, and evaluation program will provide the basis for adaptive management of agricultural drainage throughout the SJRB.

D. QUALIFICATIONS

The team members include UCB, LBNL, and UCD personnel all of whom have worked in the SJRB for the past five to ten years. The UCB group has specialized in developing tools for the analysis of microbial community structure, function and dynamics in selenium impacted environments. The UCB group has also developed X-ray absorption spectroscopy tools for the ***in situ*** determination of selenium species and distribution in microbial biomass. The LBNL group has specialized in SJRB selenium fate and transport experiments. LBNL has also developed fate and transport models to support real-time adaptive management of selenium loading. The UCD group has specialized in developing tools for the analysis of algal community structure, function and dynamics in selenium impacted environments. The UCD group has developed GC/MS tools for the determination of selenium species and distribution in algal biomass. The UCD group is recognized for their ability to assess selenium foodchain transfer characteristics and ecotoxic risk.

Professor Terrance Leighton (Microbiology and Biochemistry, UCB)

Professor Leighton has been a faculty member at UC Berkeley for the past twenty five years. He directs the UCB Bioremediation, Education, Science and Technology Center. Professor Leighton is an expert in microbial biology, microbial ecology, the molecular mechanisms that regulate hazardous metal detoxification and biosorption in bacteria, and the microbial ecophysiology of wastewater treatment systems and damaged environments.

ADMINISTRATIVE POSITIONS:

Director UCB Bioremediation Education Science and Technology Center

Founding Member - European Science Foundation Phytoremediation Scientific Network

CoDirector UCB - CalEPA Bioremediation Validation and Certification Laboratory

Dr. Nigel Quinn (Geological Scientist, **ESD,** Lawrence Berkeley National Laboratory)

Dr. Nigel Quinn was lead hydrologist in the San Joaquin Valley Drainage Program, retaining a faculty affiliation with Cornell with responsibility for development of groundwater and drainage models to support the Drainage Program's planning effort. With the sunset of the Drainage Program he has continued his work with the US Bureau of Reclamation dividing his time between monitoring efforts in support of the Grasslands Bypass project, development of real-time forecasting tools **for** the San Joaquin River and selenium fate and transport research projects. He has been affiliated with Lawrence Berkeley National Laboratory for the past 9 years. Nigel is the author of over SO publications and reports on various aspects of water resources and drainage engineering.

Dr. Teresa Fan (Associate Research Professor, UCD)

Dr. Teresa W-M. Fan is faculty member in the Department of Land, Air and Water Resources, University ***of*** California, Davis. Her research interest has been in the broad area of environmental biochemistry ranging from plant stress biochemistry and Se biogeochemistry in relation to ***in situ*** bioremediation, to mechanisms of aquatic ecotoxicity of agricultural and industrial discharges. Along CalFed's interest, she has been working on salinity and toxic metals stress on the Asian clam, *Potumocorbulu amurensis*, in the Delta/San Pablo Bay, as well as the tradeoffs between algal phytoremediation and ecotoxic risk of selenium in San Joaquin Valley's evaporation ponds. She has served on the 9-member EPA Peer Consultation Workshop on Selenium Aquatic Toxicity and Bioaccumulation (March 1998) which concluded that selenium organic forms and foodchain biochemistry - not total Se - should be the target of ecotoxic investigations and bioremediation goal. Most recently, she was one of the authors of the Central Valley Drainage Implementation Program's comprehensive report on Discharge to the San Joaquin River.

Dr. Richard Higashi (Assistant Research Professor, **UCD)**

Dr. Richard M. Higashi is a faculty member in the Crocker Nuclear Laboratory, University of California, Davis. He has worked in broad areas of environmental chemistry, ranging from toxicity identification in complex effluents such as pulpmill and oil production discharges, to DOE waste contamination remediation, to agricultural water, soil, and

sediment problems of the Central Valley and San Francisco Bay/Delta, as well as air pollution (PMIO and ozone) research in the Central Valley and Sierra Nevada Range. The chemistry of humics and other organic matter plays a central role in all of these research areas, and he is currently engaged in organic matter chemistry investigations in relation to selenium ecotoxic remediation in evaporation ponds of the **SJV.**

F. COST

1. Budget

Budget Costs: (2 year duration): Total cost: $485,000

TASK NO.	UCB	LBNL	UCD	TRAVEL / SUPPLIES / EQUIPMENT
Task 1	105,000	36,000	88,000	72,000
Task 2	71,000	80,000		45,000
TOTALS	176,000	116,000	88,000	115,000

* See attached detailed budget page.

2. Cost sharing

The Bureau *of* Reclamation, CALFED (CALFED Project B273), Exxon Corporation, SSRL and the US Army Corps of Engineers have provided previous funding which supported collection of the preliminary data cited in this proposal. The project will have access to Atomic Absorption Spectroscopy and Biolog instrumentation in the UCB BEST facilities. Professor Leighton is the PI of a DOE grant from the SSRL for XAS speciation of selenium in environmental samples by XANES and EXAFS. SLAC beam time will be used for Selenium speciation of CALFED microbiota samples. The University of California provides a portion of Professor Leighton's salary. A portion of Dr Quinn's time is paid for by the US Bureau of Reclamation in support of the Grassland Bypass Project.

G. LOCAL INVOLVEMENT

The proposed project supports a comprehensive plan to establish a real-time monitoring and water quality forecasting system in the San Joaquin Basin including all the major east-side tributaries, the west-side agricultural water districts and the main stem of the San Joaquin River. The project will contribute crucial biological data and new water quality monitoring tools to this effort. The project will support the documentation, validation and accreditation of a selenium removal biotreatment plant located in the Panoche Water District. Using ecophysiological modeling data developed by the UCB group, the treatment system has consistently reduced >80% of the selenium and >90% of the nitrate loading from an agricultural drainage wastestream.

H. COMPLIANCE WITH STANDARD TERMS AND CONDITIONS

Selenium has been identified by the SJRMP Executive Council as a water quality stressor of concem in the San Joaquin River for all species of anadromous fish and well as for wildfowl in San Joaquin Basin wetlands. Management of wetland drainage discharges through scheduling of releases to coincide with periods of San Joaquin River assimilative capacity can improve San Joaquin River water quality. CALFED is currently funding **two** projects dealing with this issue. No systematic data collection program has been undertaken to date to evaluate the role of microbiota in selenium fate and transport within the SJRB. These datasets are crucial to developing knowledge-based strategies for real-time drainage management. Such a data collection program would also support adaptive management options that are integral to current SJRMP and CALFED-sponsored initiatives on real-time water quality management in the San Joaquin River and with the Vemalis Adaptive Management Program (VAMP), a multi-agency experiment to improve the San Joaquin River fishery through manipulation of tributary flows and flow release schedules.

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ATTACHMENTS

UNIVERSITY OF CALIFORNIA, BERKELEY

BERKELEY • **DAVIS** • IRVINE • LOS ANGELES • RIVERSIDE • SAN DIEGO • SAN FRANCISCO

SANTA BARBARA • **SANTA** CRUZ

DEPARTMENT OF MOLECULAR ***AND*** CELL BIOLOGY

401 BARKER HALL
BERKELEY, CALIFORNIA **94720-3202**
FAX (510) 643-5035

May 15, 2000

Ms. Lydia Beiswanger, Chief Deputy
Merced County Board of Supervisors
2222 **M** Street
Merced, CA 95340

Dear Ms. Beiswanger:

This letter is to inform you of our intent to submit a proposal to the CALFED Bay-Delta Program entitled **"Use** of Microbial Community Profiles for Selenium Hazard. Assessment and for Management of Real-Time Electrical Conductivity and Dissolved Oxygen Sensor Biofouling". If successful in our application we intend to conduct this project at sites within the Grasslands Drainage Basin and at gauging stations such as e Mud Slough at Gustine and at Crows Landing on the San Joaquin River.

Agricultural and wetland water districts within the county spend tens of thousands **of** dollars each year on selenium analysis to monitor supply water and drainage **return** flows. This project addresses a critical knowledge gap in ecosystem understanding and management: the role of the microbiota, which form the base of the ecosystem food web, in affecting selenium fate and transport. This project proposes the isolation, characterization, analysis and monitoring of microbial communities contained in agricultural drainage and wetland return flows generated within the Grasslands Drainage Basin on the west-side of the San Joaquin Valley and in the San Joaquin River.

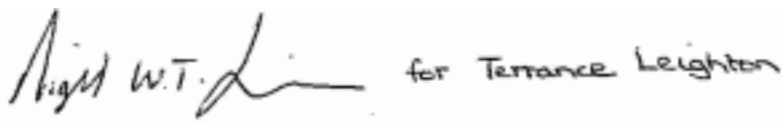
The bioaccumulation and biotransformation of selenium by these SJDS microorganisms will be studied in controlled laboratory environments, free flowing aquatic ecosystems and engineered biological treatment systems. The processes controlling the fate and nature of selenium assimilation by microbiota will be elucidated. Advanced environmental measurement methods will be developed to determine directly the distribution and chemical species of selenium present in representative microbiota. Experimental systems will be developed to evaluate the bioavailability of microbially incorporated selenium to higher trophic levels of the food chain. Near real-time biomonitoring systems will **be** developed to "fingerprint" seasonal and treatment system changes in microbiota community structure and function. The results from this project will fill crucial data gaps in our understanding of the role of microbiota in controlling selenium dynamics in the **SJDS.** The project results will also provide more realistic concentration objectives for the **SJR** and its major tributaries that will both be more protective of the environment and allow agriculture to make'use of the true assimilative capacity of the **SJR.** The current selenium objectives **are** neither seasonal nor site specific and hence are inherently inefficient.

The project will complement the current CALFED sponsored Real-Time Water Quality Management project, the long term goal of which is to expand forecasting to cover both

selenium and boron in addition to electrical conductivity and dissolved oxygen. The sensors required for this effort are increasingly compromised by microbiota biofouling. Characterization of the microbiota responsible for these sensor failures and development of appropriate decontamination strategies is crucial to the reliability and success of the Real-Time Water Quality Management project.

We anticipate considerable interest in these projects by water districts and wetland managers in Merced County.

Sincerely,



Terrance Leighton
Professor of Biochemistry and Molecular Biology

2001 PSP Preliminary Budget Table.

Please **note** that *the salary and* **benefits estimates** are **subject** to confirmation **by** the UC Berkeley Sponsored Projects **Office**

Table 1. Sample annual and total budget.

Year	Task			Direct Labor *Hours*	Subject to Overhead: *Salary*	*Benefits*	Travel	Supplies & Expendables	Service Contracts	Overhead (show % here)	Exempt from Overhead: Equipment	Graduate Student Fee Remission	Total Cost
Year 1	Task 1: Microbial community structure	UC Berkeley	Leighton	80	56,150	53,250	52.500	59,500		**51%**	$41.500		582,900
			GSRA	**960**	$18.200	58,400	52,000	52,500		51%		512,000	$43,100
	Task 1 : Ecosystem characterization	Berkeley Lab	Quinn	80					$16,500				516.500
			Research Asst.	240					56,500				56.500
	Task 1 : Eco-risk foodchain transfer	UC Davis	**Fan**	**160**					$25.500				525,500
			GSRA	**600**					$21,000				521.000
	Task 2: *Biofouling characterization*	UC Berkeley	Leighton	**80**	56,150	53,050	52,500	55.500			511.500		529,700
			GSRA	480	$7.800	$3,200	51,800						512,800
	Task 2 : Biofouling remedial actions	**Berkeley** Lab	Quinn	**160**					547,250				547,250
			Research Asst.	240					$13,000				513.000
Total Cost Year 1					538.300	517,900	58.800	518,500	$129,750	102%	553.000	512.000	5278.250
Year 2	**Task** 1: Microbial community structure	UC Berkeley	*Leighton*	**80**	55.150	$3,250	$2,500	59,500		51%			521,400
			GSRA	***960***	518,200	58,400	$2,000	52.500		51%		512,000	543,100
	Task 1 : Ecosystem characterization	Berkeley Lab	Quinn	80					$11,500				511.500
			Research Asst.	240					56.500				***$6,500***
	Task 1 : **Eco-risk** foodchain transfer	UC Davis	**Fan**	160					$20,500				$20.500
			GSRA	**800**					521,000				521.000
	Task?: Biofouling characterization	UC Berkeley	Leighton	80	56.150	53,050	52.500	56,500					518,200
			GSRA	480	57,800	$3,200	51,800						512.600
	Task 2 : Biofouling remedial actions	Berkeley Lab	Quinn	**160**					$38,750				538.750
			Research Asst.	240					513,000				513,000
Total Cost Year 2					$38,300	517,900	$8,800	$18.500	$111.250		$0	$12,000	5205,750
Total Project Cost					576.600	535,800	$17,600	$37,000	5241.000		$53.000	524,000	1486.000

From: Chris Eacock
To: Quinn. Nigel @ LBL; Quinn. Nigel @ MP
Date: 5/12/00 4:47PM
Subject: Algae biofouling of EC sensors

To whom it may concern:

The Bureau of Reclamation has made a significant commitment to collect accurate water quality data to monitor the effects of the Grassland Bypass Project (GBP) on Mud Slough and the San Joaquin River.

Between April and August, 1999, several EC sensors failed at GBP Station B, which is a crucial water quality monitoring site in the San Luis Drain. Failure of the sensors prevented real-time monitoring of water in the drain and required frequent visits to the site by USGS staff.

The problem may have been caused by algae on the sensors

Reclamation is concerned that this problem may occur with other EC sensors in the region. Therefore, we endorse any research that will identify the conditions in which the problem is manifested, the forms of algae involved, and economical solutions.

M. Chris Eacock
Natural Resource Specialist
South-Central California Area office
Bureau of Reclamation
2666 North Grove Industrial Drive, Suite 106
Fresno, California 93727-1551
559.487.5133
559.487.5130 fax
559.779.9613 cell
ceacock@mp.usbr.gov

CC: Delamore, Michael

STATE OF CALIFORNIA -- THE RESOURCES AGENCY GRAY DAVIS, *Governor*

DEPARTMENT OF WATER RESOURCES
SAN JOAQUIN DISTRICT
3374 EAST SHIELDS AVENUE
FRESNO, CA 93726-6913

May 12, 2000

Mr. Nigel Quinn
U.S. Bureau of Reclamation
Mid-Pacific Regional Office
2800 Cottage Way
Sacramento, California 95825-1898

Dear Mr. Quinn:

The San Joaquin District of the Department of Water Resources measures flow and water quality in streams throughout the San Joaquin Valley. Through the course of these activities, the District has had to deal with complications arising from algal growth and other biofouling of instream sensors and probes. Biofouling can increase *coats* associated with water quality sampling, by increasing operation and maintenance costs.

The District recognizes the need for further investigation into the problem of biofouling of instream sensors and probes, and supports this research.

Sincerely,

Paula J. Landis

Paula Landis, Chief
San Joaquin District

Environmental Compliance Checklist

All applicants must fill out this Environmental Compliance Checklist. Applications must contain answers to the following questions to be responsive and to be considered for funding. *Failure to answer these auestions and include them with the application will result in the application being considered nonresponsive and not considered for funding.*

1. **Do** any of the actions included in the proposal require compliance with either the California Environmental Quality Act (CEQA), the National Environmental Policy Act (NEPA), or both?

   _____ **YES** X **NO**

2. If you answered yes to # **1,** identify the lead governmental agency for CEQMNEPA compliance.

   _____
   Lead Agency

3. **If you answered no to # 1, explain why CEQA/NEPA compliance is not required for the actions in the proposal.**
   Proposal includes laboratory research and field monitoring.

4. **If** CEQMNEPA compliance is required, describe how the project will comply with either or both of these laws. Describe where the project is in the compliance process and the expected date ***of*** completion.

5. **Will** the applicant require access across public or private property that the applicant does **not** own to accomplish the activities in the proposal?

   _____ **YES** X NO

   If yes, the applicant must attach written permission for access from the relevant property owner(s). Failure to include written permission for access may result in disqualification of the proposal during the review process. Research and monitoring field projects for which specific field locations have not been identified will be required to provide access needs and permission for access with **30** days of notification of approval.

6. Please indicate what permits or other approvals may be required for the activities contained in your proposal. Check all boxes that apply.

LOCAL		
Conditional use permit	—	
Variance	—	
Subdivision Map Act approval	—	
Grading permit	—	
General plan amendment	—	
Specific plan approval	—	
Rezone	—	
Williamson Act Contract cancellation	—	
Other _____ (please specify)	—	
None required	X	
CESA Compliance	—	(CDFG)
Streambed alteration permit	—	(CDFG)
CWA § 401 certification	—	(RWQCB)
Coastal development permit	—	(Coastal Commission/BCDC)
Reclamation Board approval	—	
Notification	—	(DPC, BCDC)
Other _____ (please specify)	—	
None required	X	
FEDERAL		
ESA Consultation	—	(USFWS)
Rivers **&Harbors** Act permit	—	(ACOE)
CWA § ***404*** permit	—	(ACOE)
Other _____ @lease specify)	—	
None required	X	

DPC = Delta Protection Commission
CWA = Clean Water Act
CESA =California Endangered Species Act
USFWS = U.S. Fish and Wildlife Service
ACOE = U.S. Army Corps of Engineers

ESA = Endangered Species Act
CDFG = California Department of Fish and Game
RWQCB = Regional Water Quality Control Board
BCDC= Bay Conservation and Development Comm.

Land Use Checklist

All applicants must fill out this Land Use Checklist for their proposal. Applications must contain answers to the following questions to be responsive and to be considered for funding. *Failure to answer these questions and include them with the application will result in the application being considered nonresponsive and not considered for funding.*

1. **Do** the actions in the proposal involve physical changes to the land (i.e. grading, planting vegetation, or breeching levees) or restrictions in land use (i.e. conservation easement or placement of land in a wildlife refuge)?

   _____ YES X NO

2. If NO to # **1,** explain what type of actions are involved in the proposal (i.e., research only, planning only).
   Proposal involves laboratory research and field monitoring

3. If YES to # **1,** what is the proposed land use change or restriction under the proposal?

4. If YES to # **1,** is the land currently under a Williamson Act contract?

   _____ YES _____ NO

5. **If** YES **to** # **1,** answer the following:

   Current land use _____
   Current zoning _____
   Current general plan designation _____

6. If YES to **#1,** is the land classified as Prime Farmland, Farmland of Statewide Importance or Unique Farmland on the Department of Conservation Important Farmland Maps?

   _____ YES _____ **NO** _____ DON'T KNOW

7. If YES to # **1,** how many acres of land will be subject to physical change or land use restrictions under the proposal?
   _____

8. **If** YES to # **1,** is the property currently being commercially farmed or grazed?

   _____ **YES** _____ **NO**

9. If YES to **#8,** what are the number **of** employeeslacre_____
   the total number of employees _____

10. Will the applicant acquire any interest in land under the proposal (fee title **or** a conservation easement)?

YES _____ NO X

11. What entitylorganization will hold the interest?_____

12. **If** YES to # 10, answer the following:

Total number *of* acres to be acquired under proposal _____
Number **of** acres to be acquired in fee _____
Number **of** acres to be subject to conservation easement _____

13. **For** all proposals involving physical changes to the land **or** restriction in land use, describe what entity **or** organization will:

manage the property _____

provide operations and maintenance services _____

conduct monitoring _____

14. **For** land acquisitions (fee title **or** easements), will existing water rights **also** be acquired?

YES _____ NO _____

15. Does the applicant propose any modifications **to** the water right **or** change in the delivery **of** ***the*** water?

YES _____ NO X

16. If YES to # 15, describe_____